

TO THE EDITOR

Non-uniform Response to Temozolomide Therapy in a Pituitary Gonadotroph Adenoma

Endocrinologically active pituitary adenomas are treated either with surgery, radiotherapy or various drugs, including dopamine agonists, long-acting somatostatin analogs, growth hormone receptor antagonists, or corticosteroid secretion inhibitors. Fully 35% to 55% of pituitary adenomas invade adjacent structures. This figure is lower in gonadotroph adenomas, less than 5% compared to other adenoma types. Clinical management of invasive adenomas is challenging, and most of them tend to recur after surgery. Hormonally active pituitary adenomas may even be resistant to combined medical, surgical, and radiotherapy treatments. In comparison, pituitary carcinomas represent 0.2% of all adenohypophysial neoplasms¹, and not only invade adjacent structures but give rise to cerebrospinal and/or systemic metastases with high mortality. The lack of response to aggressive pituitary adenomas, particularly carcinomas to conventional therapies, drives search for new approaches¹.

Temozolomide (TMZ) is a chemotherapeutic agent which can cross the blood-brain barrier and has proven utility in the treatment of glioblastoma. More recently, it has been used to treat aggressive pituitary adenomas and carcinomas². To date, cases of such aggressive pituitary tumors have been reported in terms of clinical outcomes. The morphologic effects of therapy have been described in only three patients². Of these, two tumors responded, both radiologically and morphologically. The first case, a prolactin cell adenoma, did not express MGMT (O⁶-methylguanine-DNA methyltransferase), a DNA repair protein that counteracts TMZ anti-neoplastic action. The second case, an

aggressive silent subtype 2 corticotroph adenoma, showed no morphological change after therapy. This tumor showed high-level immunoreexpression of MGMT by immunohistochemistry. The third case, a corticotroph adenoma in a patient with Cushing's disease, showed no MGMT immunoreexpression, and 80% reduction in tumor volume was noted on magnetic resonance imaging (MRI) scan. The Ki-67 labeling index decreased after the therapy.

Herein, we report a recurrent pituitary gonadotroph adenoma in which the partial response to TMZ appeared to result from non-uniform MGMT immunoreexpression, portions of the tumor lacking immunoreactivity and the remainder showing high-level immunostaining.

CASE REPORT

This 50-year-old male patient underwent five surgeries due to recurrent pituitary adenoma since 1992. He received radiation therapy after the first surgery (50Gy). A second surgery was performed in 1995. The first and second surgical specimens were not available for morphologic studies. The surgical specimen after the third procedure in 2000 revealed a histologically diffuse and pseudorosette-forming pituitary adenoma immunoreactive for FSH and LH. Mitoses were rare and no pleomorphism was noted. Ultrastructurally, a predominantly oncocyctic adenoma consisting of rather small, closely apposed cells with infrequent immature secretory granules was observed. A diagnosis of gonadotroph adenoma was made. Immunostaining for MGMT of the 2000 specimen revealed two distinct regions, one with no expression of MGMT and the other with 60% immunopositivity. As the residual tumor increased in size, another transsphenoidal surgery was performed in 2005. The tumor was fibrotic and grossly firm, and only a minimal portion could be resected.

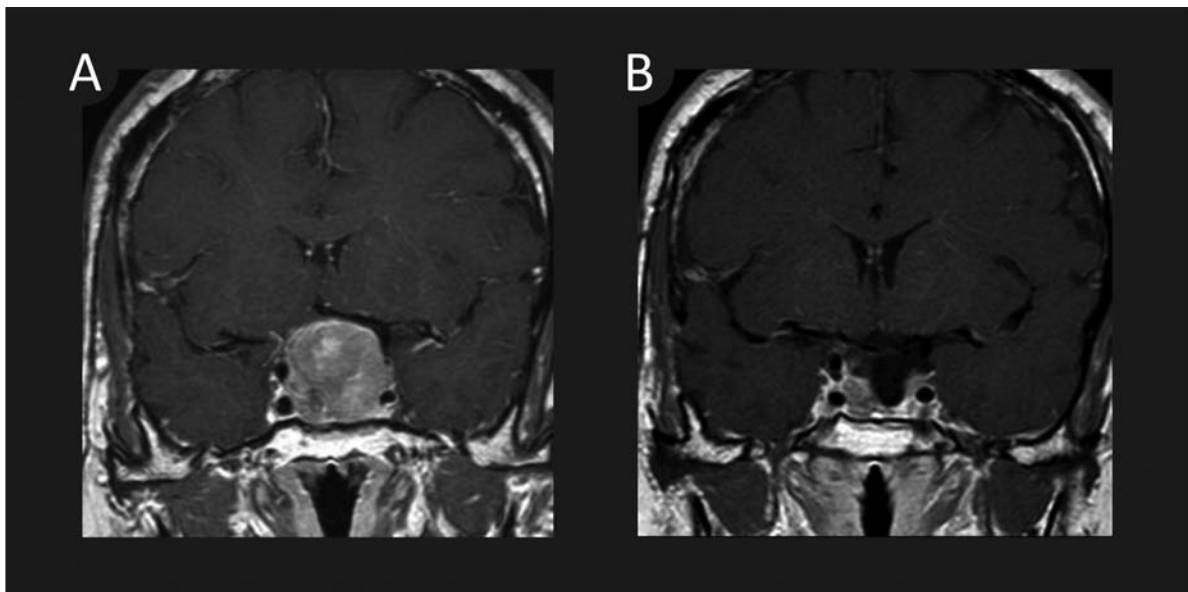


Figure 1: Coronal T1-weighted MRI with gadolinium enhancement showing a sellar and suprasellar tumor with optic chiasm compression (A). Postoperative coronal T1-weighted MRI with gadolinium enhancement disclosing a small residual tumor (B).

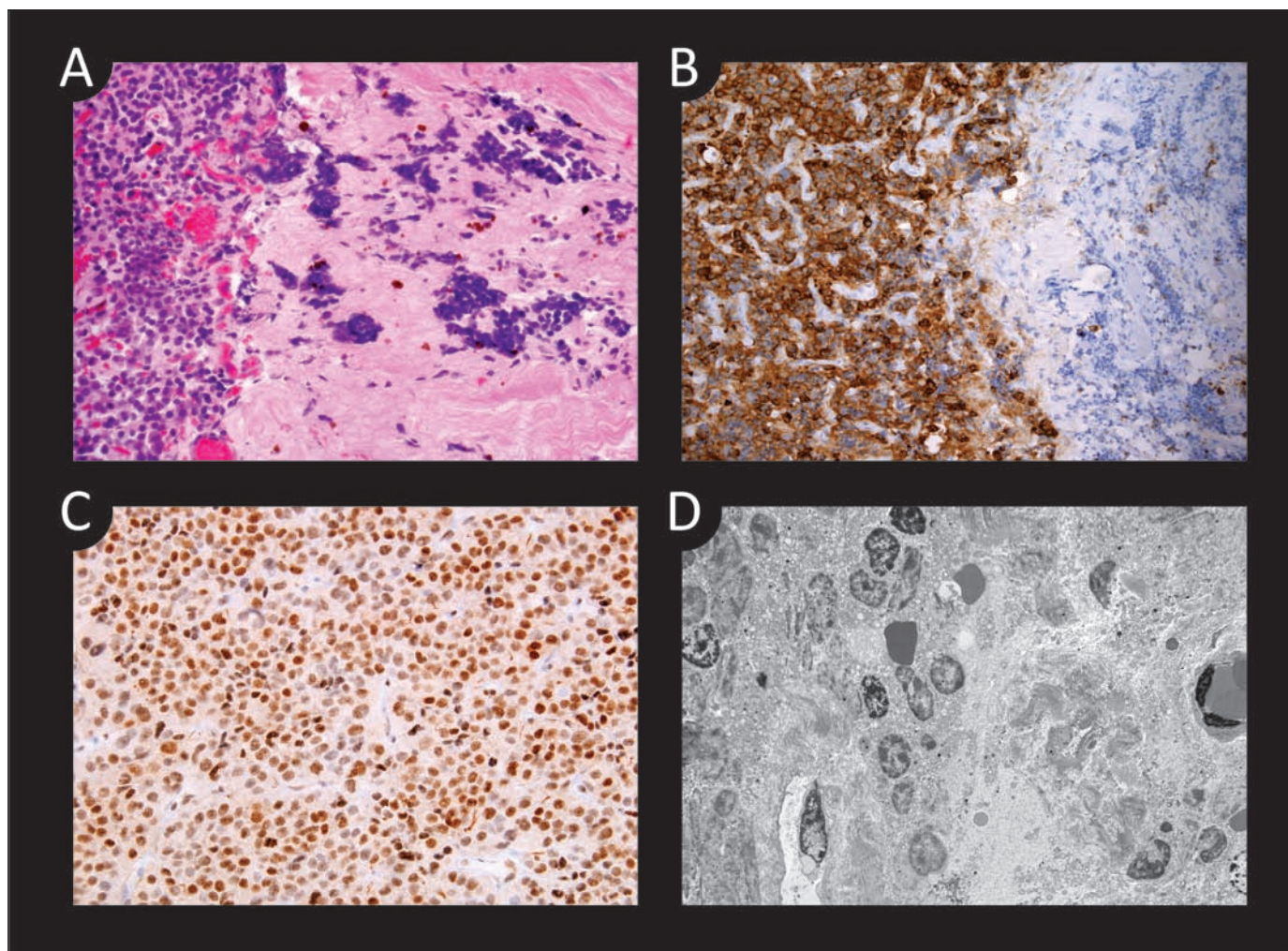


Figure 2: Slightly acidophilic pituitary adenoma. No major cellular and nuclear pleomorphism is noted. In one portion, severe cellular injury is noted. The tumor cells are shrunken, possessing a dark, chromatin rich nucleus and a narrow rim of chromophobic cytoplasm. There is marked accumulation of connective tissue. Hematoxylin & Eosin stain. Original magnification: 100x (A). The injured small cells in the fibrotic area are LH immunonegative. The surviving areas show cells with conclusive LH immunopositivity. Immunostaining for LH. Original magnification: 100x (B). The majority of the tumor cell nuclei are immunopositive for MGMT. Immunostaining for MGMT. Original magnification: 250x (C). Severe cellular damage is apparent. Electron micrograph. Original magnification: 2500x (D).

Microscopically, fragments of tumor consisted of small LH-immunoreactive cells. Mitotic activity was low and immunostaining for MGMT gave negative results.

In 2009, the patient presented with visual deterioration. An MRI scan disclosed tumor regrowth (Figure 1A). Temozolomide was started in June 2009 at a standard dose and regimen (200 mg/m²/day, 5/28) for 14 months. The tumor stabilized and the patient showed clinical improvement, although MRI did not demonstrate changes. This was followed by reoperation in August 2010 in order to obtain tumor cytoreduction. Grossly, the lesion was softer, more friable, and subtotal resection was performed (Figure 1B). Postoperative evolution was uneventful. Temozolomide was restarted after surgery at the same regimen, and it has been continued until the present time. Recent MRI disclosed a stable residual lesion.

MORPHOLOGICAL FINDINGS

Microscopically, the recurrent gonadotroph adenoma showed severe cellular damage (Figure 2A). The severely damaged tumor cells showed no LH immunoreactivity (Figure 2B). Fibrosis was prominent. Mitoses were not observed, and the Ki-67 labeling index was 2%. The majority of tumor cell nuclei were immunopositive for MGMT (Figure 2C). Ultrastructurally, several tumor cells showed severe injury (Figure 2D). In summary, this gonadotroph adenoma, having a low mitotic index but a high recurrence rate, showed a non-uniform MGMT immunoreactivity, a feature apparently underlying the heterogeneous response to treatment.

DISCUSSION

Treatment approaches to aggressive pituitary adenomas include surgery, radiotherapy, medical therapy and chemotherapy¹.

Temozolomide, a second generation alkylating chemotherapeutic agent, readily crosses the blood-brain barrier and exerts its cytotoxic effect through methylation of DNA at the O⁶ position of guanine, which then mispairs with thymine during the next cycle and inhibits all phases of tumor cell growth. Predicted clinical outcomes of TMZ treatment in non-resistant tumors are growth arrest, reduction in tumor volume, tumor cell damage, and control of biochemical abnormalities.

MGMT is a DNA repair protein that reverses alkylation at the O⁶ position of guanine by transferring the alkyl group to a sulfur group by cysteine within its sequence. By removing alkylating adducts induced by the therapy, it counteracts the antineoplastic effect of TMZ. The few reported studies of TMZ therapy of pituitary adenomas showed that resistant tumors have high-level MGMT immunoreactivity³.

The heterogeneous immunoreactivity of MGMT observed in our case raises questions regarding the efficacy of TMZ therapy. The tissue obtained after TMZ therapy showed areas with zones of tumor cell destruction as well as non-damaged, MGMT-immunoreactive cells. The clinical improvement noted after TMZ therapy, therefore, may be due to response of the MGMT-negative portion of the tumor, thus relieving the mass effect and the optic chiasm compression even if the tumor had not showed MRI changes. The consistency of the tumor was different after TMZ treatment with more soft and friable areas, which could be the result of the MGMT-negative portions to TMZ exposure, thus facilitating its cytorreduction. This observation has been noted previously⁴.

It is controversial whether MGMT immunoreactivity can predict tumor response to TMZ. Several studies support the inverse correlation between low-level MGMT immunoreactivity and TMZ response³. Other studies, however, suggest that MGMT analysis has no predictive value⁵. Other than MGMT immunoreactivity, the MGMT promoter status can also be evaluated by methylation specific polymerase chain reaction (MS-PCR)⁵. The reliability of which, on paraffin-embedded tissue, is controversial. Our tumor demonstrating heterogeneous MGMT immunoreactivity showed a partial response to TMZ. The viable MGMT immunopositive tumor cells may represent the TMZ-resistant portion of the tumor, whereas many immunonegative tumor cells seem to have undergone necrosis due to the cytotoxic effects of TMZ. This is the first case of a pituitary tumor morphologically studied after TMZ treatment showing that heterogeneity.

Another question arises about how long to continue the treatment. Some reports show tumor recurrence and resistance to TMZ after the therapy was discontinued¹. In our case, we decided to continue TMZ in order to avoid possible resistance and recurrence. The tumor reported in the present paper is an

uncommon example of gonadotroph adenomas exhibiting aggressive behavior despite low proliferative activity but invading surrounding structures and recurring several times.

ACKNOWLEDGMENT

This manuscript is dedicated to the bright memory of Dr. Bernd W. Scheithauer. The authors are grateful to the Jarislowsky and Lloyd Carr Harris Foundations for their continuous support. The authors thank Mrs. Denise Chase of Mayo Clinic for her excellent assistance and secretarial support.

Ayca Ersen
Dokuz Eylul University, Izmir, Turkey

Luis V. Syro, Luis C. Penagos
Hospital Pablo Tobon Uribe and Clinica Medellin
Medellin, Colombia
Email: lvsyro@une.net.co

Humberto Uribe
Clinica SOMA, Medellin, Colombia

Bernd W. Scheithauer (deceased)
Mayo Clinic, Rochester, MN, USA

Leon D. Ortiz
Instituto de Cancerologia,
Clinica Las Americas, Medellin, Colombia

Fabio Rotondo, Eva Horvath, Kalman Kovacs
St. Michael's Hospital. University of Toronto
Toronto, Ontario, Canada

REFERENCES

1. Heaney AP. Clinical review: Pituitary carcinoma: difficult diagnosis and treatment. *J Clin Endocrinol Metab.* 2011;96(12):3649-60.
2. Ortiz LD, Syro LV, Scheithauer BW, et al. Temozolomide in aggressive pituitary adenomas and carcinomas. *Clinics (Sao Paulo).* 2012;67 Suppl 1:119-23.
3. McCormack AI, Wass JA, Grossman AB. Aggressive pituitary tumours: the role of temozolomide and the assessment of MGMT status. *Eur J Clin Invest.* 2011;41(10):1133-48.
4. Syro LV, Uribe H, Penagos LC, et al. Antitumour effects of temozolomide in a man with a large, invasive prolactin-producing pituitary neoplasm. *Clin Endocrinol.* 2006;65(4):552-3.
5. Raverot G, Sturm N, de Fraipont F, et al. Temozolomide treatment in aggressive pituitary tumors and pituitary carcinomas: a French multicenter experience. *J Clin Endocrinol Metab.* 2010;95(10):4592-9.